AN ARGINASE INHIBITOR(S) AND ITS POSSIBLE ROLE IN THE DEVELOPMENTAL DECREASE OF ARGINASE ACTIVITY IN CHICK EMBRYOS[1]

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A number of enzyme activities have been measured in crude homogenates of developing embryos, and in several instances changes in activity have been correlated with morphological events and the time tissues and organs begin to function: *e.g.* cholinesterase in amphibian embryos (Sawyer, 1943). Such correlations suggest that enzyme activities measured in crude homogenates reflect events involved in metabolic differentiation. Consequently, it seems reasonable to believe that studies of mechanisms by which enzyme activities change will also yield information concerning mechanisms involved in differentiation at the biochemical level. With this assumption in mind we began studying arginase activity in developing chick embryos in an attempt to establish the cause of the decrease in activity per unit moist weight which occurs during development (Needham and Brachet, 1935; Fisher and Eakin, 1957; Clark and Fischer, 1957; Roeder, 1957).

At the outset of these studies we assumed that the arginase activity expressed by crude homogenates is primarily a measure of the amount of enzyme present. Evidence obtained in the course of surveying activity in various tissues led us to question this assumption. Subsequently, we observed that several tissue homogenates which exhibit little or no arginase activity are capable of inhibiting the activity of embryo homogenates. Preliminary studies indicate that inhibition is due to the presence of a high molecular weight inhibitor(s) and are consistent with the possibility that it is ribonucleic acid in nature. The presence of an arginase inhibitor(s) in chick tissues raises the possibility that the developmental decrease in activity could be due to an increase in the concentration of this agent as well as a decrease in the concentration of enzyme.

Materials and Methods

Eggs from a flock of Rhode Island Reds were incubated at 39° C. in a commercial incubator. Eggs were removed at various times, cracked in a pan of water and the entire living area excised. The vitelline membrane was removed and embryo, pellucida, vasculosa and vitellina were separated. At the 24-hour stage no vascular area is present; however, a pre-vascular area exists, containing mesodermal tissue (Hamilton, 1952) which we refer to as vascular area. At several ages, area vitellina and area vasculosa were separated into two layers: ectoderm and endoderm in the case of vitellina, somatopleure and splanchnopleure in the case of vasculosa. The various tissues were weighed and homogenized in distilled

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water in the cold with a pestle homogenizer having a teflon pestle and a glass vessel. Homogenates were assayed for arginase activity using the procedure described below.

Enzyme was activated by heating 4 ml. of homogenate with 0.2 ml. of a 20 per cent solution of $MnCl_2 \cdot 4H_2O$ for 20 minutes at 57° C. Activated homogenate was equilibrated to 30° C. in a water bath and 1 ml. of an 18 per cent solution of arginine hydrochloride at pH 9.5 was added. A saturated solution of NaOH was used to adjust the pH of the arginine solution. One-ml. samples of reaction mixture were removed at 0, 20, 40 and 60 minutes after arginine addition. The samples were heated to 90° C. for 10 minutes to stop enzymatic activity. Two and one half ml. of 0.2 *M* phosphate buffer at pH 6.8 were added to each sample

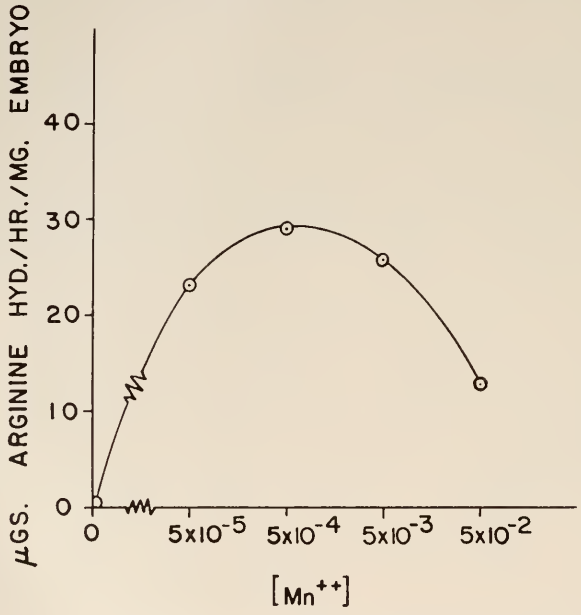

FIGURE 1. Effect of Mn^{++} concentration on arginase activity of 4-day embryo homogenate (15 mg. per ml. reaction mixture).

and 0.2 ml. of a glycerol urease preparation from jack bean meal (Folin and Wu, 1919) was used to hydrolyze urea present. After incubation with urease for 10 minutes at room temperature, 2 ml. of half-saturated Na_2CO_3 were added and the ammonia transferred to 0.05 N H_2SO_4 by a stream of ammonia-free air. Nessler's reagent (Folin and Wu, 1919) was added to the acid solutions and the color read on a Klett-Summerson colorimeter, using filter number 54. Ammonia nitrogen present was calculated from a standard curve prepared with analytical grade ammonium sulfate. Nitrogen values for the sample inactivated immediately after arginine addition were subtracted from all values and the corrected values were plotted against time of incubation with arginine. The slope of the resulting curve was used as a measure of arginase activity. In this report activities are presented as μg. arginine hydrolyzed per hour per ml. reaction mixture or per mg. tissue.

Activation of arginase

The procedure outlined above gives a 5×10^{-2} *M* concentration of Mn^{++} in the activation mixture. Van Slyke and Archibald (1946) and Greenberg (1955) recommend this concentration to activate liver arginase. We have found that this concentration gives full activity for some but not all chick embryo tissues, *e.g.* 4-day embryo homogenates give maximum activity with 5×10^{-4} *M* Mn^{++} (Fig. 1). In surveying activity changes during development and in experiments involving tissue mixtures, it seemed desirable to use the same concentration throughout. Consequently, 5×10^{-2} *M* Mn^{++} was almost always used. In some experiments involving 4-day embryos 5×10^{-4} *M* Mn^{++} was used. These cases will be noted in the text. Also, $MnSO_4$ in maleate buffer (Greenberg, 1955) was used to activate and found to give the same result as $MnCl_2$ in distilled water.

Van Slyke and Archibald (1946) recommend heating to 57° C. for 20 minutes in the presence of Mn^{++} to activate arginase in crude liver homogenates. Arginase appears to be stable under these conditions. We have found that partially purified liver arginase gives the same activity whether heated or not. Greenberg (1955) recommends heating to 60° C. for 20 minutes as one step in arginase isolation. It should be noted that heating in the absence of Mn^{++} destroys all activity of homogenates (Fig. 1).

Incubation with substrate

The procedure outlined above gives a final arginine concentration of 0.17 *M* in the reaction mixture. A study has been made of 4-day embryo arginase activity at various substrate concentrations. These results give an apparent K_m of 8×10^{-3} *M* which is in good agreement with values of 11.6 and 7.7×10^{-3} reported for liver arginase (Greenberg, 1951).

Most tissues have been assayed at two or more concentrations and in all cases activity appears to be linear with concentration (Figure 5 contains data for 4-day embryo activated at 5×10^{-4} *M* Mn^{++}).

The pH of reaction mixtures prepared as described above is 9.2. Activities have been measured at several hydrogen ion concentrations and maximum activity was found at pH 10, which is in agreement with results reported by Greenberg (1951).

Measurement of urea formed

Phosphate buffer addition to heated samples as described previously gives a pH of 7.5. Urea present has been shown to be quantitatively hydrolyzed by treatment with urease under these conditions. Added urea can be recovered quantitatively from reaction mixtures as ammonia (± 2 μg. N per ml. reaction mixture). When urease is omitted from the procedure, no arginase activity is observed. Arginase activities can be duplicated on the same homogenate, ± 2 μgs. urea nitrogen or ± 12 μg. arginine hydrolyzed per hour per ml. reaction mixture. When no arginase activity was observed, a value was calculated consistent with the sensitivity of the assay (12 μg. arginine hydrolyzed per hour per ml. reaction mixture).

Results

Distribution of arginase activity in developing eggs

The first objective of this study was to describe the changes in arginase activity of several tissues in eggs during the first 7 days of development. Embryo, pellucida, vasculosa and vitellina were excised, weighed and homogenized. An aliquot was removed for dry weight determination. Before the living area was dissected, the distance from the center to the outer edge of pellucida, vasculosa and vitellina was measured (several measurements were made on each tissue in each egg and the averages recorded). From these studies we could follow changes in the moist weight, dry weight, surface area and arginase activity per unit moist weight, dry

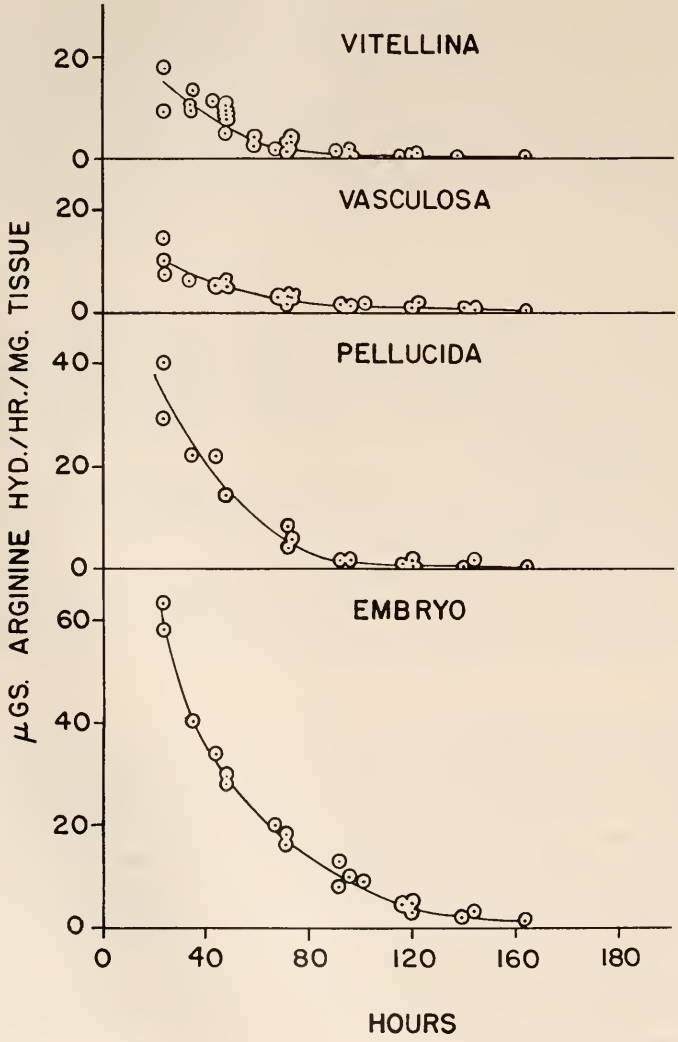

Figure 2. Arginase activities of various tissues in developing eggs.

TABLE 1

Arginase activity of tissue layers in yolk sac of developing eggs

Incubation time of eggs—hours	Mg. ectoderm per mg. vitellina	μg. arginine hydrolyzed per hour per mg. tissue			
		Ectoderm	Endoderm	Vitellina	Calculated activity of vitellina
64	0.38	8.2	1.2	1.3	3.9
73	0.37	9.8	2.0	2.6	4.9
65	0.35	13.9	0.0 (<0.9)	1.8	4.8
	Mixture of homogenates—35% ectoderm			1.8	4.8
98	0.33	4.5	0.0 (<2.0)	1.7	1.5
	Mg. somatopleure per mg. vasculosa	Somatopleure	Splanchnopleure	Vasculosa	Calculated activity of vasculosa
73	0.25	8.7	0.0 (<0.5)	1.7	2.2
144	—	1.0	0.0 (<0.3)	0.0 (<0.2)	—

weight and surface area. Embryos increased in moist weight in a pattern almost identical with that reported by Schmalhausen (1926). Pellucida increased in moist weight from 2 mg. at the first day to 300 mg. on the seventh day, vasculosa increased from 10 to 800 mg. and vitellina increased from 46 on the first day to 300 on the third day and subsequently decreased to 180 mg. on the seventh day. Surface areas of pellucida, vasculosa and vitellina followed essentially the same pattern as moist weight, *i.e.*, pellucida and vasculosa increased in area from the first to the seventh day and vitellina increased to a maximum on the third day. An aliquot of each homogenate was dried at 60° C. for two days in a petri dish and the residue weighed. Per cent dry weight was essentially constant in each tissue during the first 7 days of development (embryo 9 per cent, pellucida 10 per cent, vasculosa 17 per cent and vitellina 13 per cent). Arginase activity decreased during development in each tissue when calculated per unit moist weight, dry weight or surface area. Activities calculated per unit moist weight are shown in Figure 2. When activity at the first day was assigned a value of 100 per cent for each tissue, it was found that the pattern of change during incubation was almost the same in all tissues. Area vitellina is of particular interest because of the way it spreads over the surface of yolk. Apparently (Hamilton, 1952), this tissue spreads by addition of cells to its outer edge, which are formed from periblastic syncytium at the zone of junction. If this picture is correct, tissue at the outer edge is younger, relative to the time cells are formed, than in the inner area. We measured arginase activity in a strip of tissue approximately 10 mm. wide around the outer edge of vitellina. From the first to the seventh day this strip exhibits substantially the same activity as the remainder of vitellina. It follows that the mechanisms which cause activity to decrease are operating in the non-cellular syncytium which becomes vitellina; otherwise, activity in the peripheral strip should not decrease as rapidly as in the remainder of vitellina. These observations suggest that all tissues in developing eggs tend to decrease in arginase activity and that the activity decrease is not correlated with any obvious developmental event.

Data in Figure 2 show that tissues exhibit a common tendency to decrease in arginase activity; however, these tissues show marked differences in their level of activity whether calculated on a moist weight or dry weight basis. It appears that there is a gradient of activity related to difference in proximity to yolk. Embryo, the most distant, exhibits the highest arginase activity; pellucida the next most distant, exhibits the next highest activity; and yolk sac tissues in contact with yolk exhibit the lowest activity. Results in Table I show a very significant difference in activity between ectoderm and endoderm of vitellina, and between somatopleure and splanchnopleure of vasculosa. In every case the tissue in contact with yolk material exhibited a much lower activity than the tissue distant from yolk. Consequently, the gradient can be extended to the tissue layers of yolk sac. Furthermore, it was possible to calculate the activity of total tissue (vitellina or vasculosa) from the weights and activities of its component layers (Table I). It was found that at approximately the third day of incubation there was a significant difference between the calculated activity of vitellina and the measured activity. In one case enough of the tissues were obtained so that homogenates of ectoderm and endoderm could be assayed together and separately. In this case endoderm homogenate clearly inhibited the activity of ectoderm. These results are consistent with the possibility that endoderm, at least at one stage, is capable of inhibiting the arginase activity of ectoderm.

Inhibition of embryo arginase activity

Results described in the preceding section suggested that endoderm of area vitellina contains an arginase inhibitor(s). Greenberg (1951) has reported that most amino acids inhibit arginase. It was conceivable that yolk sac tissues contain relatively large amounts of free amino acids from digestion of yolk proteins. An attempt was made to demonstrate inhibitor activity in endoderm using embryo homogenates as sources of arginase activity (Table II). Endoderm homogenates were dialyzed in an effort to remove possible low molecular weight inhibitors (Table II). Dialysis was carried out in the cold against distilled water. Results show that endoderm homogenates do inhibit but that dialysis does not remove inhibitory activity as would be expected if the agents were amino acids. Furthermore, casein hydrolysate, obtained commercially, does not inhibit at a dry weight concentration equal to that of endoderm which is inhibitory. From these results we concluded that inhibition of embryo arginase by endoderm homogenates is not due to free amino acids in the endoderm. It should be noted that dialysis actually increases the inhibitory activity of endoderm homogenates. An explanation of this effect will be presented in a later section of this report.

Preliminary studies of the nature of the inhibitor(s) have been undertaken, using vitellina of eggs incubated 4 days as a source of inhibitor. The dialysis effect described above has been confirmed with vitellina. An attempt was made to destroy inhibitory activity by heating to 90° C. for 20 minutes. Results obtained show that this treatment does not destroy inhibitory activity but actually increases inhibition. These results suggest that the inhibitor(s) is a high molecular weight, heat-stable substance. Moss (1952) reported that sodium ribonucleate from yeast inhibits arginase. We have confirmed this observation, using 4-day embryo as a source of arginase activity. The first two steps of a ribonucleic acid

(RNA) isolation procedure were applied to area vitellina homogenate (Kay and Dounce, 1953). Inhibitory activity was found in the fraction which, in the case of mammalian liver, contains RNA. Four-day vitellina was homogenized in a solution of NaCl and sodium citrate. The homogenate was centrifuged, yielding a supernatant and precipitate (ppt. 1). The supernatant was adjusted to pH 4.5 with HCl and centrifuged, yielding ppt. 2 and supernatant which was adjusted to pH 7.0 with NaOH for testing (final supernatant). The two precipitates were suspended in distilled water. These three fractions were assayed for arginase

TABLE II

Inhibition of embryo arginase by endoderm homogenates

Incubation time of eggs—hours	Mg. per ml. reaction mixture		μg. arginine hydrolyzed per hour per mg. embryo
	Embryo	Endoderm*	
70	5.4	0	14
	5.4	2	10
	5.4	3	8.0
	5.4	2 (dial. 1 day)	0 (<2)
	5.4	3 (dial. 1 day)	0 (<2)
96	15	0	7.9
	15	140	4.6
	15	140 (dial. 1 day)	0.8
	15	140 (dial. 4 days)	0 (<0.8)
	15	140 (dial. 7 days)	0 (<0.8)
140	40	0	3.9
	40	9	3.4
	40	12	2.8
	40	9 (dial. 1 day)	1.6
	40	12 (dial. 1 day)	1.2
161	175	0	0.90
	175	125	0.50

* Endoderm homogenates did not exhibit arginase activity alone at the concentrations used in any of these experiments.

activity and arginase inhibitor activity using 4-day embryo homogenate as a source of arginase activity. Only ppt. 1 exhibits arginase activity and only ppt. 2 inhibits embryo arginase. Mixtures of ppt. 1 and embryo in three assays yielded higher activity than predicted. This result can be explained by the presence of an arginase stimulatory agent in embryo. Evidence for the existence of such an agent will be presented in a later report.

These preliminary studies indicate that the inhibitor(s) is high molecular weight in nature and are consistent with the possibility that it is RNA.

Distribution of inhibitor

The presence of inhibitor(s) in tissues which do not exhibit arginase activity can be demonstrated by showing that homogenates inhibit the arginase activity of

4-day embryos, as was done with area vitellina and area vitellina endoderm. It was found that heart from 21-day embryos does not exhibit arginase activity and inhibits 4-day embryo arginase (Fig. 3). As in the case of vitellina and vitellina endoderm, dialysis for 4 days in the cold against distilled water increases the inhibitory activity of heart homogenate (Fig. 3). Heart from 6-week-old chickens also inhibits 4-day embryo arginase. Results obtained show this inhibition when enzyme is activated with 5×10^{-2} or 5×10^{-4} M Mn^{++}.

In order to directly demonstrate the presence of inhibitor(s) in a tissue which exhibits arginase activity, it is necessary to destroy its arginase activity before testing against 4-day embryo arginase. It is known that dialysis inactivates

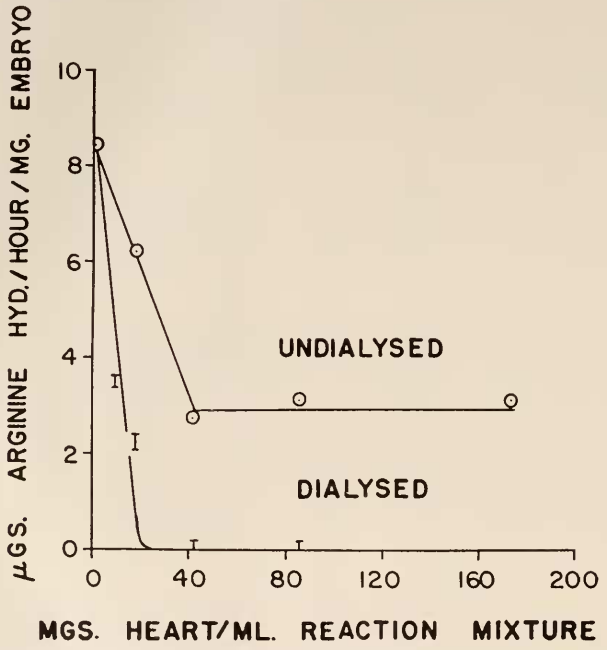

FIGURE 3. Effect of 21-day embryo heart homogenate on the arginase activity of 4-day embryo (15 mg. per ml. reaction mixture). Dialysis was carried out for 4 days in the cold against distilled water.

arginase (Greenberg, 1951). We have confirmed this observation, using partially purified beef liver arginase which was obtained commercially. Activation with Mn^{++} under the conditions we are using does not restore activity. We have destroyed the arginase activity of several tissue homogenates in this manner and tested the dialyzed homogenates for inhibitor activity, using 4-day embryos as a source of arginase activity. It was observed that homogenates of bone marrow, skin, leg muscle, eye and brain from 21-day embryos can completely inhibit the arginase activity of 4-day embryos. The effect of dialyzed brain homogenate is shown in Figure 4. These results clearly show that all tissues tested contain arginase inhibitor(s), if the only effect of dialysis is to inactivate arginase. The observation was made with vitellina, vitellina endoderm and heart that dialysis

increases the inhibitor activity of tissue homogenates which express little or no arginase activity. This is inconsistent with the above interpretation unless these tissues do contain arginase which does not exhibit activity because of the presence of inhibitor. Evidence is presented in the following section that heart does contain arginase even though crude homogenates do not exhibit arginase activity. Earlier it was shown that heating vitellina homogenate to 90° C. for 20 minutes, which inactivates arginase, also increases inhibitor activity. These results are consistent with the interpretation that the primary effect of dialysis is to inactivate arginase.

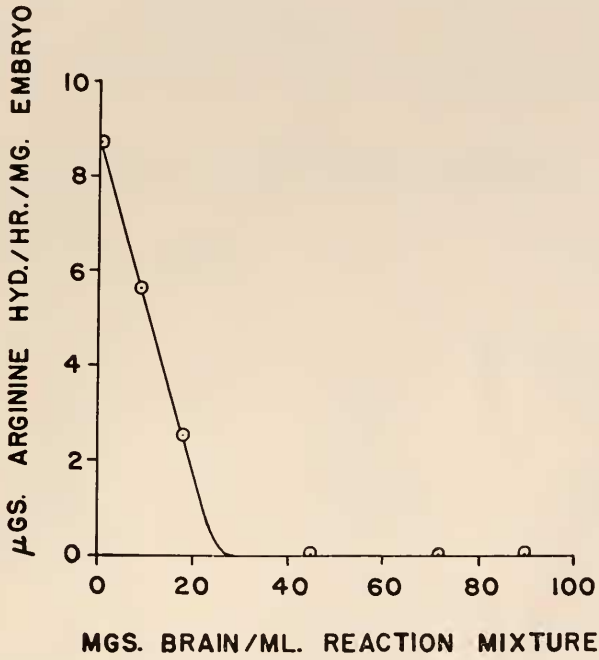

FIGURE 4. Effect of dialyzed brain (from 21-day embryos) homogenate on the arginase activity of 4-day embryo (15 mg. per ml. reaction mixture). Dialysis was carried out for 4 days in the cold against distilled water.

Role of inhibitor in establishing the arginase activity of crude homogenates

If inhibitor(s) is playing a major role in establishing the arginase activity expressed by homogenates, it is necessary that inhibitor(s) be effective against enzyme in the same tissue as well as against 4-day embryo arginase. Homogenate of heart from 6-week-old chickens has been shown to inhibit 4-day embryo arginase. It was found that 8-day embryo heart exhibits arginase activity and that heart from 6-week-old chickens inhibits this activity. A second type of evidence is that dialyzed 4-day embryo homogenate inhibits the arginase activity of undialyzed 4-day embryo homogenate. From these results we believe it is reasonable to assume that inhibitor(s) is effective against enzyme in the same tissue as well as 4-day embryo arginase.

If inhibitor(s) is playing a major role in establishing the activity of crude homogenates, it is necessary that tissues containing inhibitor(s) contain more enzyme than is indicated by the activity of crude homogenates. We attempted to test this possibility by partially isolating arginase from heart of 6-week-old chickens, which does not exhibit arginase activity as a crude homogenate. The arginase isolation procedure recommended by Greenberg (1955) for horse liver was applied to heart (Table III). These results show that heart contains at least 30-fold more enzyme than is indicated by the activity of crude homogenate. It appears that acetone precipitation "unmasks" arginase activity, presumably by inactivating inhibitor since the acetone-soluble fraction does not inhibit 4-day embryo arginase. A similar case has been reported with a pantothenic acid-requiring mutant of Neurospora (Wagner and Guirard, 1948; Wagner, 1949).

TABLE III

Partial isolation of arginase from hearts of 6-week-old chickens

Isolation step	Fraction	Per cent original N	μg. arginine hydrolyzed per hour per	
			Mg. original tissue	Mg. N in fraction
Heart homogenate incubated overnight with Mn^{++} at pH 7.6	extract A	100	0 (<0.02)	0 (<3)
Acetone precipitation	soluble	6	0.03	81
	insoluble	84	0.62	118
Extraction of acetone Powder with buffer	insoluble	66	0.15	37
	extract B	11	0.26	370
Extract B heated at 60° C. for 20 minutes	precipitate	3	0 (<0.01)	0 (<31)
	extract C	5	0.24	810
Extract C dialyzed and lyophilized	powder D	1.4	0.19	2180

Non-growing pads and homogenates of wild type are capable of coupling pantoyl lactone and beta-alanine to form pantothenic acid. Such preparations of the mutant do not show this activity; however, acetone powders of both organisms can catalyze the reaction. As in the case of arginase, acetone precipitation "unmasks" an enzyme activity.

Possible role of inhibitor(s) in the developmental decrease of arginase activity

Evidence has been presented in a previous section that area vitellina contains an arginase inhibitor(s) and that arginase activity in this tissue decreases during development in a manner similar to other tissues, *e.g.* embryo. Inhibitor activity has been demonstrated in all tissues tested. Results presented in the previous section indicate that inhibitor(s) is playing a role in establishing the activity of crude homogenates. Consequently, the developmental decrease of activity could

be the result of an increase in the concentration of inhibitor(s), a decrease in the concentration of enzyme, or the concentration of both agents could change, the net result being a decrease in arginase activity. Even if the concentration of inhibitor remains constant, its presence would cause activity to decrease more than would be warranted by a decrease in enzyme concentration, since there would be an increase in the inhibitor(s)-to-enzyme ratio. The developmental decrease in arginase activity would accurately represent a decrease in enzyme concentration only in the event that enzyme exhibits a *constant* fraction of its activity in various tissues during the developmental period studied. If the modifying influence of homoge-

TABLE IV

Arginase activity of mixtures of 21-day embryo tissues with 4-day embryo

Tissue	Mg. per ml. reaction mixture		μg. arginine hydrolyzed per hour per		
			Ml. reaction mixture		Mg. tissue
	Tissue	Embryo	Experimental	Calculated	
Brain	0	20	180		9.0
	90	20	118	298	
	90	0	118		1.3
Leg muscle	0	10	81		8.1
	82	10	69	150	
	82	0	69		0.84
Eye	0	20	149		7.5
	45	20	193	286	
	45	0	137		3.0
Bone marrow	0	10	69		6.9
	36	10	69	88	
	36	0	19		0.53
Skin	0	10	56		5.6
	63	10	124	125	
	63	0	69		1.1

nates on the activity of arginase is constant in various tissues, mixtures of tissues should exhibit an activity equal to the sum of the activities of the tissues measured separately. Homogenates of several tissues from 21-day embryos were mixed with homogenate of 4-day embryo and the mixtures assayed for arginase activity (Table IV). From these results we concluded that mixtures of tissue homogenates do not necessarily exhibit an activity equal to the sum of the activities of the tissues measured separately. Only in the case of skin was activity equal to the calculated value. Apparently, embryo does not exhibit any activity in the presence of brain or leg muscle. These results are not consistent with the possibility that the modifying influence of all homogenates is the same. Consequently, we believe that the different arginase activities expressed by various tissue homogenates can not be explained simply by differences in enzyme content.

Results described in Table IV can be explained with the assumption that tissue homogenates are effectively mixtures of enzyme and inhibitor(s), provided the enzyme-inhibitor interaction is reversible. Evidence for reversibility has been obtained, using 4-day embryo as a source of arginase activity and heart from 6-week-old chickens to inhibit. Results presented in Figure 5 show that this system exhibits the properties which, according to Ackermann and Potter (1949), are characteristic of reversible inhibition. If tissue homogenates are effectively mixtures of enzyme and a reversible inhibitor(s), mixtures should exhibit activities equal to or less than the sum of the components but equal to or greater than the

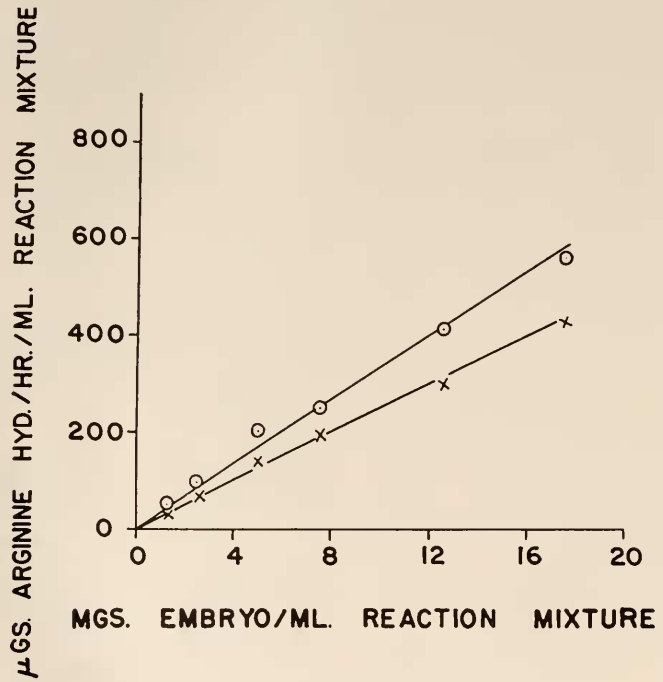

FIGURE 5. Effect of heart from 6-week-old chickens (120 mg. per ml. reaction mixture) on the arginase activity of 4-day embryo at various concentrations. Activated with 5×10^{-4} *M* Mn^{++}. ⊙ embryo alone, X plus heart.

activities of the least active components. Results in Table IV are consistent with these requirements. If the activity of a tissue homogenate is determined by the presence of a reversible inhibitor as well as enzyme, it is possible that activity would not be proportional to tissue concentration. Data in Figure 5 show that the arginase activity of 4-day embryos is proportional to tissue concentration over a 10-fold range. Such a proportionality has been shown by Straus and Goldstein (1943) when the ratio of inhibitor concentration to dissociation constant is greater than 100 (zone c). Our results can be explained on the basis of zone c inhibition.

Assuming that homogenates are effectively mixtures of enzyme and inhibitor(s), the data in Table IV require that different tissues have different inhibitor(s)-

to-enzyme ratios. Consequently, enzyme does not exhibit the same fractional activity in all tissues and, therefore, inhibitor(s) must be playing some role in the differentiation process which results in each tissue having its characteristic arginase activity, as measured in homogenates. This suggests that the developmental decrease in activity could also be at least in part due to a change in inhibitor concentration. We have found that heart arginase activity decreases between the eighth and the twenty-first day of incubation. At the twelfth day, heart exhibits a small but significant arginase activity and does not inhibit 4-day embryo arginase. At the twenty-first day, heart does not exhibit a measurable arginase

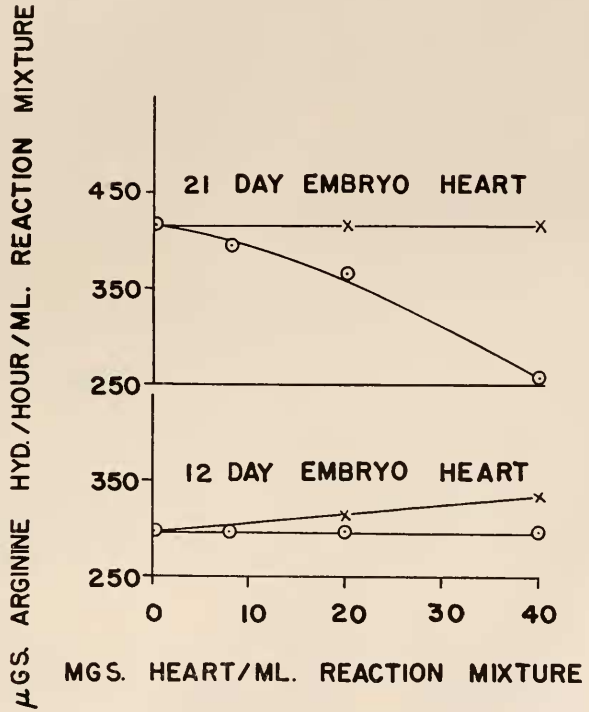

FIGURE 6. Effect of heart from 12- and 21-day embryos on the arginase activity of 4-day embryos (12.5 mg. per ml. reaction mixture) activated with 5×10^{-4} *M* Mn^{++}. ⊙ embryo-heart mixture, X embryo activity + heart activity as measured separately.

activity and inhibits 4-day embryo arginase (Fig. 6). These results show that a decrease in arginase activity is correlated with an increase in the ability to inhibit embryo arginase.

Evidence presented in this section suggests that the arginase activity expressed by tissue homogenates is determined by the concentrations of both enzyme and inhibitor(s), different tissues contain different relative amounts of inhibitor to enzyme and that in one case the developmental decrease of arginase activity is correlated with an increase in inhibitory activity. We believe these results demonstrate that inhibitor(s) plays some role in the developmental change in arginase activity.

Discussion

Results reported here suggest that ribonucleic acids could be inhibiting arginase activity in crude chick embryo homogenates. Sorm and Hrubesova (1955) found that ribonucleic acid inhibits chymotrypsin and trypsin; Moss (1952) found that ribonucleic acids inhibit arginase; Klingenberg (1952) reported that cathepsin is inhibited by ribonucleic acid; Chepinoga and Pavlovskii (1956) found that ribonucleic acid and deoxyribonucleic acid inhibit aldolase and enolase; and Zittle (1946) reported that succinic dehydrogenase is inhibited by both deoxyribonucleic acid and ribonucleic acid. A large number of cases have been reported where proteins inhibit enzyme activities (*e.g.* Swartz, Kaplan and Frech, 1956). These numerous instances where high molecular weight inhibitors have been found in various tissues and organisms raise the possibility that enzyme activities of crude homogenates are not necessarily a measure of enzyme content.

Changes in the enzymic activity of developing embryos, as measured in crude homogenates, have been demonstrated in numerous instances and it seems reasonable to believe that these changes constitute one major aspect of differentiation. It would be of considerable interest to identify the mechanisms that are responsible for these observed changes. Before we can understand such mechanisms, we must establish the meaning of the change in activity. It is conceivable that an enzyme activity, as measured in crude homogenates, could change during development in at least three ways: (1) in the amount of enzyme per unit tissue, (2) in the nature of the enzyme, and (3) in the modifying influence of substances present in the homogenate. Markert (1958) has obtained evidence that embryos produce different esterases at different stages of development. Results presented in this report suggest that in the case of arginase, substances which modify its activity may play a role in the developmental change. Consequently, it is clear that the nature of the change which is expressed as a change of enzymic activity must be established before an attempt can be made to determine the mechanism causing the change.

Summary

1. Arginase activity was measured in tissues of developing eggs from the first to the seventh day of incubation. Activity per unit moist weight or dry weight decreased in embryo, pellucida, vasculosa and vitellina.

2. Evidence was obtained that all chick tissues studied contain a high molecular weight arginase inhibitor(s) which appears to be ribonucleic acid in nature.

3. Inhibitor(s) seems to play a major role in establishing the level of arginase activity expressed by crude homogenates.

4. The decreased arginase activity of hearts from developing embryos has been correlated with an increase in inhibitor activity.

5. These results suggest that the developmental decrease in arginase activity could be the result of an increase in inhibitor concentration as well as of a decrease in enzyme concentration.

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